

# Validation of the sulphur hexafluoride (SF<sub>6</sub>) tracer gas technique for measurement of methane and carbon dioxide production by cattle

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Boadi, D. A., Wittenberg, K. M. and Kennedy, A. D. 2002. **Validation of the sulphur hexafluoride (SF<sub>6</sub>) tracer gas technique for measurement of methane and carbon dioxide production by cattle.** *Can. J. Anim. Sci.* **82**: 125–131. Methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) production from six crossbred yearling beef heifers (400 ± 13.0 kg) were measured, using the sulphur hexafluoride (SF<sub>6</sub>) tracer gas technique (Tracer) and open-circuit hood calorimetry (Cal) to validate the former in estimating rumen CH<sub>4</sub> and CO<sub>2</sub> production in the field. Animals were individually fed a diet consisting of 50% barley concentrate and 50% alfalfa cubes at 1.3 × maintenance requirements daily. Heifers were divided into two groups for individual animal 24-h gas measurements by each method. Each group of heifers was rotated between the Cal and Tracer techniques for 6 consecutive days in an incomplete block design. Methane production ranged from 108 to 145 L d<sup>-1</sup> (mean 130 ± 4.0 L d<sup>-1</sup>) using the Cal technique, and 90 to 167 L d<sup>-1</sup> (mean 137 ± 4.0 L d<sup>-1</sup>) using the Tracer technique. The mean CH<sub>4</sub> production (L d<sup>-1</sup>) was not different ( $P = 0.24$ ) between the two methods. Carbon dioxide production with the Tracer technique was 20% higher than CO<sub>2</sub> production with the Cal technique ( $P < 0.01$ ). The range of CO<sub>2</sub> production was 1574 to 2049 L d<sup>-1</sup> (mean 1892 ± 74.0 L d<sup>-1</sup>) by Cal, and 1541 to 3330 L d<sup>-1</sup> (mean 2353 ± 74.0 L d<sup>-1</sup>) by Tracer. Day-to-day variation in CH<sub>4</sub> production was not different within each method ( $P > 0.05$ ); however, animal-to-animal variation (11.7%) was significant for the Tracer technique ( $P = 0.04$ ), but not for the Cal technique ( $P = 0.53$ ). Comparison of the equality of variance between the two methods showed that there were no differences in variations ( $P > 0.05$ ) between Cal and Tracer for CH<sub>4</sub> production. On the other hand, variations in CO<sub>2</sub> production were not equal ( $P > 0.05$ ) between methods. Day-to-day variation in CO<sub>2</sub> production was significant using Cal, but not Tracer ( $P > 0.05$ ). Animal-to-animal variation in CO<sub>2</sub> production was 1.6 and 11.8% by Cal and Tracer techniques, respectively. It can be concluded that the SF<sub>6</sub> tracer technique accurately estimated rumen CH<sub>4</sub> production, but CO<sub>2</sub> production was 20% higher. The study suggests that for CH<sub>4</sub> measurements using the SF<sub>6</sub> tracer technique, more animal numbers are needed than for Cal to reduce animal-to-animal variation.

**Key words:** Methane, carbon dioxide, SF<sub>6</sub> tracer technique, validation, cattle

Boadi, D. A., Wittenberg, K. M. et Kennedy, A. D. 2002. **Validation de la technique de mesure du méthane et du dioxyde de carbone produits par les bovins par traçage à l'hexafluorure de soufre (SF<sub>6</sub>).** *Can. J. Anim. Sci.* **82**: 125–131. Les auteurs ont mesuré la quantité de méthane (CH<sub>4</sub>) et de dioxyde de soufre (CO<sub>2</sub>) libérée par six génisses de boucherie hybrides d'un an (400 ± 13,0 kg) par la technique du traçage à l'hexafluorure de soufre (SF<sub>6</sub>) puis ont validé cette technique en estimant le volume de CH<sub>4</sub> et de CO<sub>2</sub> produit par le rumen au champ avec une hotte calorimétrique à circuit ouvert. Chaque animal a reçu une ration composée à 50 % de concentré d'orge et à 50 % d'agglomérés de luzerne correspondant à 1,3 × le régime d'entretien quotidien. Les génisses ont été réparties en deux groupes et le volume de gaz libéré par les animaux de chaque groupe a été déterminé au moyen d'une des deux méthodes. Chaque méthode a été appliquée successivement aux deux groupes pendant 6 jours d'affilée dans le cadre d'une expérience en blocs incomplets. Les animaux libèrent de 108 à 145 litres de méthane par jour selon la technique calorimétrique (moyenne de 130 ± 4,0 litres par jour) et de 90 à 167 litres selon la technique du traçage (moyenne de 137 ± 4,0 litres par jour). Le volume moyen de CH<sub>4</sub> (litres par jour) était identique ( $P = 0,24$ ) dans les deux cas. La quantité de CO<sub>2</sub> obtenue par traçage dépassait celle établie par calorimétrie de 20 % ( $P < 0,01$ ). La production de CO<sub>2</sub> variait de 1 574 à 2 049 litres par jour (moyenne de 1 892 ± 74,0 litres par jour) pour la technique calorimétrique et de 1 541 à 3 330 litres (moyenne de 2 353 ± 74,0 litres par jour) pour celle du traçage. La quantité de CH<sub>4</sub> libérée ne varie pas beaucoup d'un jour à l'autre, quelle que soit la méthode ( $P > 0,05$ ). Toutefois, elle varie sensiblement d'un animal à l'autre (11,7 %) avec la technique du traçage ( $P = 0,04$ ), mais pas avec la technique calorimétrique ( $P = 0,53$ ). Quand on compare la variance des deux méthodes, on se rend compte qu'il n'y a pas d'écart ( $P > 0,05$ ) entre la variation du volume de CH<sub>4</sub> déterminée avec l'une ou l'autre méthode. Le volume de CO<sub>2</sub>, en revanche, fluctue ( $P > 0,05$ ) avec la technique. Ainsi, la quantité de dioxyde de carbone varie sensiblement d'une journée à l'autre avec la technique calorimétrique, mais pas avec celle du traçage ( $P > 0,05$ ). La calorimétrie et le traçage saisissent respectivement 1,6 % et 11,8 % de la variation du volume de CO<sub>2</sub> d'un animal à l'autre. On en conclut que le traçage au SF<sub>6</sub> permet d'estimer avec précision la production de CH<sub>4</sub> par le rumen, mais fausse celle de CO<sub>2</sub> de 20 %, à la hausse. L'étude donne à penser qu'il faudrait plus d'animaux pour mesurer le volume de CH<sub>4</sub> avec la technique du traçage qu'avec la calorimétrie si l'on veut atténuer la variation d'un animal à l'autre.

**Mots clés:** Méthane, dioxyde de carbone, traçage au SF<sub>6</sub>, validation, bovins

**Abbreviations:** ADF, acid detergent fibre; CP, crude protein; DM, dry matter; DMI, Dry matter intake; GE, gross energy; GEI, Gross energy intake; NDF, neutral detergent fibre

Methane production by cattle has often been measured using respiration calorimetry (Young et al. 1975; McLean and Tobin 1987), from which a number of prediction equations and models have been derived to estimate CH<sub>4</sub> production (Blaxter and Clapperton 1965; Holter and Young 1992; Wilkerson et al. 1995). Respiration calorimetry allows gases respired and eructed into a hood or chamber to be monitored and quantified, from which heat production of animals can be indirectly assessed (McLean and Tobin 1987). Respiration calorimetry involves restricting animals from their natural environment, in order to estimate gas production, thus making it inapplicable to grazing animals. As a result, information on CH<sub>4</sub> production of grazing ruminants exposed to variable environmental conditions and feed selection is limited.

The SF<sub>6</sub> tracer gas technique, which was designed for free-moving animals, allows direct rumen CH<sub>4</sub> measurement without restricting animals from their natural environment and feeding behaviour (Johnson et al. 1994). Also, measurements can be made simultaneously on several animals. The tracer gas technique uses an inert non-toxic gas, SF<sub>6</sub>, as a marker. The tracer gas, released at a known rate in the rumen, allows the concentrations of gases eructed and respired from the mouth and nose to be quantified. The tracer can account for changes in dilution of expired gases associated with head or air movements, and can therefore be used in variable wind speeds (Johnson and Johnson 1995). The SF<sub>6</sub> tracer gas technique, however, does not account for the proportion of hindgut CH<sub>4</sub> (11%) that is lost through the anus (Murray et al. 1976), and animals must be trained to wear a gas collection apparatus. There are variable reports on validation studies involving the SF<sub>6</sub> tracer gas technique and respiration calorimetry (Johnson et al. 1994; Ulyatt et al. 1999). Some of the differences in the reports can be attributed to measurements made with respiration calorimetry using the chamber, which accounts for rumen and all hind gut CH<sub>4</sub> losses, while the SF<sub>6</sub> tracer gas technique does not account for all the hindgut losses. There is a need to confirm that the SF<sub>6</sub> tracer gas technique can measure rumen CH<sub>4</sub> production, and to establish the degree of variability expected.

There is evidence that the majority of eructed gases from the rumen (over 90%) are inhaled via the trachea into the lungs before exhalation to the atmosphere along with respiratory gases (Colvin et al. 1957; Hoernicke et al. 1965; Young and Corbett 1972). It is, therefore, proposed that the SF<sub>6</sub> tracer technique also has the potential to measure the rate of CO<sub>2</sub> production. The determination of CO<sub>2</sub> production rates by the SF<sub>6</sub> tracer technique has not previously been considered, and thus would require validation. Prediction of heat production from CO<sub>2</sub> production rates would allow energy balance studies in grazing animals using the SF<sub>6</sub> tracer technique.

The objectives of this study were to validate the SF<sub>6</sub> tracer technique in measuring CH<sub>4</sub> and CO<sub>2</sub> exhaled by cattle, with respiration calorimetry using the ventilation hood (which measures respired gases). A second objective was to establish the degree of variability associated with the SF<sub>6</sub> tracer technique measurements.

## MATERIALS AND METHODS

### Animals and Management

Six crossbred yearling beef heifers (400 ± 13.0 kg) (mean ± SD) were used to compare CH<sub>4</sub> and CO<sub>2</sub> production rates using the open-circuit calorimetry hood (Cal) and the SF<sub>6</sub> tracer gas technique (Tracer). The study was conducted at the Laird McElroy Environmental and Metabolic Centre at the University of Alberta Research Farm, Edmonton, Alberta, from 8 to 21 August 1999.

Animals were fed individually once daily, a diet that consisted of 50% rolled-barley concentrate and 50% alfalfa cubes at 1.3 × maintenance requirements (Table 1). Dry matter intake of the diet ranged from 4.4 to 4.8 kg d<sup>-1</sup> among animals. Animals were chosen from a previous experiment in which animals consumed the same diet as shown in Table 1 for 3 mo. They were adjusted to their ration allotment for 1 wk prior to the start of gas collection. The level of feeding ensured that all feed was consumed and that intake relative to body weight was similar for all animals. Water was provided ad-libitum. Feeding began at 0800 h, after which animals were moved from pens for gas sampling using both methods. Animals were cared for in accordance to the guidelines of the Canadian Council on Animal Care.

### Experimental Design and Treatments

Gas was collected from each animal using each method for 3 d. Since a limited number of animals could be sampled by the calorimetry system, animals were randomly divided into two groups (three animals each). Alternating for 6 d, one group of animals went into the ventilation hoods for calorimetry, and the other group of animals were put in an open ventilation facility (with a roof) with individual stalls for Tracer gas collection. The open ventilation facility was used for Tracer collection to prevent the build-up of background gases from contaminating individual heifer air samples. Gas was sampled simultaneously for 24 h using both methods, shortly after feeding when all animals had adjusted to their respective methods. The experiment was conducted as an incomplete block design, with days as blocks.

### Methane and CO<sub>2</sub> Determination by Calorimetry

Methane and CO<sub>2</sub> production were determined using the open-circuit calorimetry system, which employed a ventilated hood that enclosed the animal's head (Young et al. 1975). Animals could stand or lie tethered in a stall. Animals were adapted to the ventilation hood in a previous experiment. Three 24-h collections were completed for each animal over the course of 6 d. Water was provided ad-libitum in the hood. Oxygen concentration was measured using a single-circuit Servomex paramagnetic oxygen analyzer (Model #540A, Crowbridge, UK). Carbon dioxide and CH<sub>4</sub> concentrations were measured with non-dispersion infrared analyzers (Model 880A, Rosemount Analytical Inc., La Habra, CA). All analyzers were calibrated at the beginning of each day's analyses as described by McLean and Tobin (1987). Air pressure and flow were recorded using a Foxboro electronic pressure and flow transmitter connected to a

**Table 1. The chemical analysis (DM basis) of diet<sup>z</sup> fed to heifers**

	Barley concentrate <sup>y</sup>	Alfalfa cubes
Crude protein (%)	15.1	16.5
Acid detergent fibre (%)	8.0	34.9
Neutral detergent fibre (%)	17.4	43.9
Ash (%)	6.6	10.9
Gross energy (kJ g <sup>-1</sup> )	18.1	17.6
Dry matter intake (kg d <sup>-1</sup> )	2.3	2.3

<sup>z</sup>The dry matter intake of diet ranged from 4.4 to 4.8 kg d<sup>-1</sup> among animals.

<sup>y</sup>Contained (DM basis): barley grain (78.4%); alfalfa-grass hay (10%); canola meal (7.0%); canola oil (2.2%); calcium phosphate (0.2%); calcium carbonate (1.3%); fortified salt (0.35%); Dynamate (0.3%) and vitamin ADE + Rumensin (0.225%).

Strawberry Tree terminal Panel T41 (Strawberry Tree Inc., Sunnyvale, CA.). The average flow rate over the 6 d of sampling was 237 ± 22.0 L min<sup>-1</sup>. Lines from the animal hoods were switched using Ascoelectric switches (Ascoelectric Ltd., Brantford ON). Methane, CO<sub>2</sub> and O<sub>2</sub> data were collected using the control setup of Workbench P.C 2.0 (Strawberry Tree Inc., Sunnyvale, CA.).

Before the experiment, a known amount of nitrogen gas was released into the calorimetry hoods to determine the recovery of the system. Nitrogen recovery factors were used to adjust CH<sub>4</sub>, CO<sub>2</sub> and O<sub>2</sub> results (Galbraith et al. 1998). The mean calorimetry room temperature as animals entered and exited was 16 ± 1.0°C and 17 ± 0.8°C, respectively.

### Methane and CO<sub>2</sub> Analyses by SF<sub>6</sub> Tracer Technique

Methane and CO<sub>2</sub> were sampled from each heifer for three 24-h periods using the SF<sub>6</sub> tracer gas technique (Johnson et al. 1994). Stainless steel permeation tubes (12.5 mm × 40 mm) were charged with 260–300 mg of SF<sub>6</sub> at liquid nitrogen temperatures, and incubated at 39°C. Predetermined release rates of SF<sub>6</sub> were achieved by measuring the weight loss of tubes for 8 wk prior to rumen insertion. Sulphur hexafluoride release rates ranged from 250 to 500 ng min<sup>-1</sup>. Based on a pre-trial test, and the study of Westberg et al. (2001), which showed the release rates of permeation tubes after removal from the rumen were similar (*P* > 0.05) to release rates prior to insertion into the rumen, the release rates of the permeation tubes were assumed constant or linear during the experimental period.

Stainless steel permeation tubes containing SF<sub>6</sub> with known release rates, were placed in the rumen per os of all heifers a week prior to the start of the experiment. This allowed enough time for the tracer gas to equilibrate in the rumen. During this period, animals were trained to wear the gas collection apparatus. Animals were moved into a chute daily for attachment and removal of collection apparatus. In the open ventilation facilities, animals were placed in stalls, and not tethered during collection. Water was provided ad libitum.

Expired gases were drawn into pre-evacuated (30 mm Hg) stainless steel collection spheres (130 mm diameter) through a 900 mm length of capillary tubing (128 µm i.d) with an in-line 15 µm filter and flexible nose piece. Collection apparatus were hung on the east and west side of the open ventilation facility each day to collect background air samples, which were used to correct expired gas concentrations. Collected gas spheres were checked for pressure to identify blocked or leaking capillary systems. Spheres were then pressurized to 110 KPa with pure N<sub>2</sub> to prevent sample contamination prior to analyses, and to allow injection of gas samples into the sample loop of a gas chromatograph. The mean minimum and maximum temperature during Tracer sampling was 9 ± 1.2°C and 22 ± 2.2°C, respectively.

A gas chromatograph (Star 3600, Varian, Mississauga, ON) fitted with an electron capture detector was used to determine SF<sub>6</sub>, and a flame ionization detector was used to determine CH<sub>4</sub> and CO<sub>2</sub> concentration in collected samples. Samples were analyzed in duplicate. The gas chromatograph was fitted with a Molecular Sieve 0.5 nm (1800 mm) column for SF<sub>6</sub> and a Poropak QS (1800 mm) for CH<sub>4</sub> and CO<sub>2</sub>. The column and injector temperatures were 35°C and 350°C, respectively, and nitrogen was used as the carrier gas with a flow rate of 30 mL min<sup>-1</sup>. Prepared standards were used to standardize the gas chromatograph for SF<sub>6</sub> (20 ppt, Scott-Marrin Inc., Riverside, CA); CH<sub>4</sub> (100 ppm; Supelco, Mississauga, ON) and CO<sub>2</sub> (1614 ppm; Matheson gas products Edmonton, AB) prior to sample analysis. Gas concentrations (SF<sub>6</sub>, CH<sub>4</sub> and CO<sub>2</sub>) were determined from peak areas and identified from their different retention times relative to the known standards.

Daily CH<sub>4</sub> and CO<sub>2</sub> production was calculated as follows (Johnson et al. 1994):

$$\begin{aligned} \text{CH}_4 \text{ (L min}^{-1}\text{)} &= \text{SF}_6 \text{ (L min}^{-1}\text{)} \times [\text{CH}_4]/[\text{SF}_6] \\ \text{CO}_2 \text{ (L min}^{-1}\text{)} &= \text{SF}_6 \text{ (L min}^{-1}\text{)} \times [\text{CO}_2]/[\text{SF}_6] \end{aligned}$$

where SF<sub>6</sub> is the predetermined release rate from the permeation tube and [CH<sub>4</sub>], [CO<sub>2</sub>] and [SF<sub>6</sub>] are the concentrations of CH<sub>4</sub>, CO<sub>2</sub> and SF<sub>6</sub> in samples after background concentrations have been deducted.

### Chemical Analyses

Feed samples were dried for 48 h in a forced draught oven at 60°C for dry matter (DM) determination. Samples were ground using a Wiley mill fitted with a 1-mm screen. Dried samples were analyzed for crude protein (CP) using a Kjeltac 1030 auto analyzer [Tecator Inc., Herndon, VI; Association of Official Analytical Chemists (AOAC) 1990, method no. 984.13], and ash, method no. 942.05 (AOAC 1990). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined using an ANKOM 200 fibre analyzer (Fairport NY), with procedures described by Komarek (1993). Gross energy (GE) was determined using a Parr 1241 adiabatic bomb calorimeter.

### Statistical Analyses

Methane and CO<sub>2</sub> production data from both methods were analyzed by the analyses of variance using GLM of the SAS

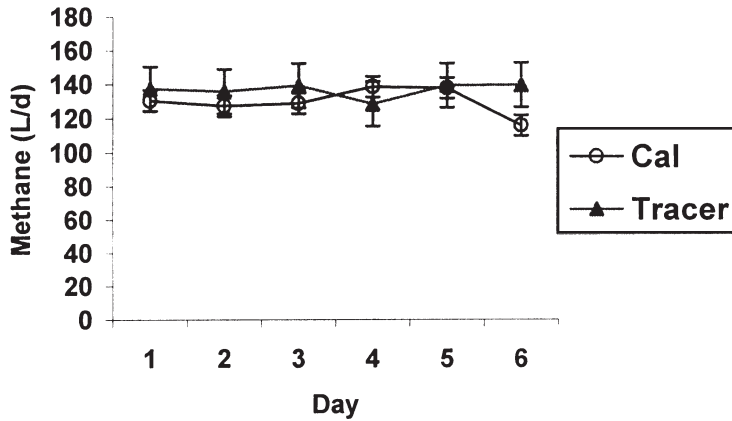


Fig. 1. Daily CH<sub>4</sub> production of heifers (mean ± SE; *n* = 3) measured by calorimetry (Cal) and SF<sub>6</sub> tracer technique (Tracer).

Institute, Inc. (1990) using the model:

$$Y_{ijk} = \mu + M_i + A_j + D_k + \epsilon_{ijk}$$

where  $Y_{ijk}$  is the trait under consideration;  $\mu$  is the overall mean;  $M_i$  is the methods with ( $i = 1, 2$ );  $A_j$  is the Animals with ( $j = 1 \dots 6$ ) and  $D_k$  is the day with ( $k = 1 \dots 6$ ); and  $\epsilon_{ijk}$  is the experimental error term. Means were separated at the 5% level of significance using the probability of differences (PDIFF) options. The effect of day was further evaluated in each method. To compute day-to-day and animal-to-animal variations within each method, Type III expected mean squares were generated for the random effects (animal and day). To compare equality of variability of CH<sub>4</sub> and CO<sub>2</sub> production by the two methods, the residual variances from each method derived in the GLM were subjected to an *F*-test (Ratio of the larger error variance:smaller error variance) at  $P = 0.05$ .

## RESULTS AND DISCUSSION

### Methane Production

Methane production ranged from 108 to 145 L d<sup>-1</sup> (mean 130 ± 4.0 L d<sup>-1</sup>) using the Cal method, and from 90 to 167 L d<sup>-1</sup> (mean 137 ± 4.0 L d<sup>-1</sup>) using the Tracer method. The mean CH<sub>4</sub> production (L d<sup>-1</sup>) was not different ( $P > 0.05$ ) between methods (Table 1). Also, there was no effect of day on CH<sub>4</sub> measurements within each method ( $P > 0.05$ ; Fig. 1). The average loss of GEI as CH<sub>4</sub> was similar by both methods ( $P > 0.05$ ; Table 1), and values were within the range reported by growing animals (Johnson et al. 1994; McCaughey et al. 1997).

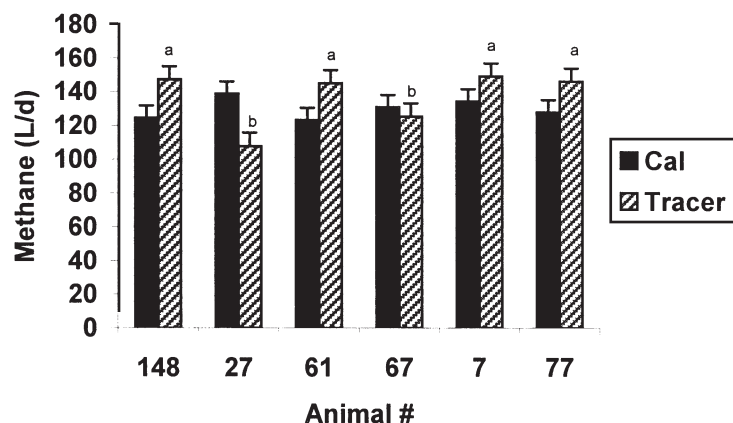
Johnson et al. (1994) showed that the SF<sub>6</sub> tracer average of 11.6 ± 0.7 L h<sup>-1</sup>, compared with 12.9 ± 0.7 L h<sup>-1</sup> calculated from respiratory chamber measurements, was about 90% of the values of chamber measurements. This is expected, as the chamber method measures both rumen and hind gut CH<sub>4</sub>, whereas the SF<sub>6</sub> tracer method and the ventilation hood method used in this study do not measure all hind gut CH<sub>4</sub>. Similarly, it was reported in an experiment using 10 sheep fed chaffed lucerne that CH<sub>4</sub> emission estimated with the SF<sub>6</sub> tracer technique was 95% of respiratory chamber estimated emissions (Ulyatt et al. 1999). On the other hand,

Ulyatt et al. (1999) reported that estimates of CH<sub>4</sub> production made in the respiration chamber (7.7 ± 0.7 L h<sup>-1</sup>) using five Friesian calves fed Rhodes grass were twice ( $P < 0.005$ ) estimates with the SF<sub>6</sub> technique made either in pens (4.1 ± 0.4 L h<sup>-1</sup>) or conducted concurrently in the chamber (4.0 ± 0.4 L h<sup>-1</sup>). Although more CH<sub>4</sub> production can be expected from calorimetry using the chamber as opposed to the ventilation hood, the lower-than-expected results of 50% more CH<sub>4</sub> from the chamber were attributed to the poor storage and transport of gases for analyses. To avoid loss and contamination of gases during transport of gases for analyses in our study, a positive pressure was created in the collected samples by pressurizing the spheres with nitrogen prior to transport. Also, gas samples were immediately analyzed upon arrival to avoid problems of leakage associated with long storage.

Average CH<sub>4</sub> production by individual animals ranged from 123 to 139 L d<sup>-1</sup> with the Cal method to 108 to 149 L d<sup>-1</sup> on the Tracer method (Fig. 2). Mean CH<sub>4</sub> production for animal no. 27 and animal no. 67 were significantly lower than for other animals using the Tracer technique. There were no differences ( $P > 0.05$ ) in CH<sub>4</sub> production among animals using the Cal method (Fig. 2).

Day-to-day variation of CH<sub>4</sub> production was not significant with the Tracer ( $P = 0.93$ ) and Cal methods ( $P = 0.20$ ). The lack of significant daily variations can be expected as the diet and amount fed were constant for all heifers in the trial. On the other hand, a significant animal-to-animal variation (11.7%) in CH<sub>4</sub> production was observed with the Tracer method, but not with the Cal method (0.1%;  $P = 0.53$ ). The higher animal-to-animal variation than observed with the Tracer method agrees with recent observations using the SF<sub>6</sub> tracer technique for CH<sub>4</sub> production measurements (Lassey et al. 1997; Leuning et al. 1998; Ulyatt et al. 1999).

Ulyatt et al. (1999) noted that, in general, when the feed intake and the composition of the diet are similar for all animals, significant between-animal differences accounted for most of the variance with a lesser amount attributed to differences among days. There is, therefore, a need to include sufficient animals to detect differences between treatments and sufficient collection days per animal to minimize between-day variation with the use of the Tracer method. In



**Fig. 2.** Comparison of heifers' CH<sub>4</sub> production measured by calorimetry (Cal) and SF<sub>6</sub> tracer technique (Tracer). *a-b* Means within a method with different letters differ ( $P < 0.05$ ).

studies with grazing animals, between-animal and between-day variations in CH<sub>4</sub> production were strongly related to the amount and composition of pasture selected (Lassey et al. 1997; McCaughey et al. 1999). Lassey et al. (1997) observed much higher variation between animals (87%) and between collection days (13%) for mature dairy cows under grazing conditions. In testing the equality of variance in CH<sub>4</sub> production between Cal and Tracer (at  $F_{8/8(0.05)}$  value = 3.44), there were no significant differences between the residual variance of the two methods ( $F = 2.09$ ). Following rigorous comparison of the traditional calorimetry technique using a ventilation hood and the SF<sub>6</sub> tracer technique, which both measure respired gases, it can be concluded that the SF<sub>6</sub> tracer technique is accurate and can be used to measure rumen CH<sub>4</sub>. However values using the SF<sub>6</sub> tracer technique need to be adjusted up in order to estimate total CH<sub>4</sub> production. The studies of Murray et al. (1976) and Johnson et al. (1994) suggest that this adjustment will be up by about 10% to account for hind gut CH<sub>4</sub> production lost through the anus.

### Carbon Dioxide Production

Carbon dioxide production ranged from 1574 to 2049 L d<sup>-1</sup> (mean  $1892 \pm 74.0$  L d<sup>-1</sup>) using the Cal method, and from 1541 to 3330 L d<sup>-1</sup> (mean  $2353 \pm 74.0$  L d<sup>-1</sup>) using the Tracer method. Tracer CO<sub>2</sub> production was 20% higher ( $P < 0.01$ ) than Cal CO<sub>2</sub> production (Table 2). It is possible that the higher activity of animals outside as a result of more opportunity for movement (non-tethered stalls) and excitement (hooking up and removal of canisters in the chute), contributed to the higher CO<sub>2</sub> production observed with the Tracer technique. Although more than 90% of rumen gases go to the lungs before exhalation, there is no information about mixing of CO<sub>2</sub> gas with the tracer gas.

Carbon dioxide production using the Tracer method was similar from day to day ( $P = 0.77$ ), whereas daily differences ( $P = 0.003$ ) were observed using the Cal method (Fig. 3). This was due to the larger variation observed with the Tracer method, and because small daily differences could not be detected. Day-to-day variations (CV = 4.1%) in CO<sub>2</sub> production were significant ( $P < 0.05$ ) with the Cal method,

**Table 2.** Methane and CO<sub>2</sub> production by heifers ( $n = 6$ ) as measured using two sampling techniques (Means  $\pm$  SE)

Trait	Calorimetry	SF <sub>6</sub> Tracer gas	<i>P</i> -value
CH <sub>4</sub> (L d <sup>-1</sup> )	130 $\pm$ 4.0	137 $\pm$ 4.0	0.24
CH <sub>4</sub> (%GEI) <sup>z</sup>	6.3 $\pm$ 0.2	6.7 $\pm$ 0.2	0.23
CO <sub>2</sub> (L d <sup>-1</sup> )	1892 $\pm$ 74.0a	2354 $\pm$ 74.0b	<0.01

<sup>z</sup>GEI = gross energy content of diet  $\times$  DMI.

*a-b* Means within a row with different letters differ ( $P < 0.05$ ).

but not with the Tracer method (CV = 0.1 %;  $P > 0.05$ ). Average CO<sub>2</sub> production by individual animals ranged from 1770 to 2022 L d<sup>-1</sup> with the Cal method, while the range was 1894 to 2749 L d<sup>-1</sup> with the Tracer method (Fig. 4). Mean CO<sub>2</sub> production of animal no. 61 and animal no. 67 was significantly lower than the other animals using the Cal technique; however, with the Tracer method no differences ( $P > 0.05$ ) were observed in CO<sub>2</sub> production among animals.

Animal-to-animal variation in CO<sub>2</sub> production was 11.8% using Tracer and 1.6% using the Cal method ( $P > 0.05$ ). In testing equality of the variance between the two methods [at  $F_{8/8(0.05)}$  value = 3.44], the residual variance for CO<sub>2</sub> production yielded significant differences ( $F = 92.3$ ), which supports the lack of equality of estimates by the two methods for CO<sub>2</sub> production. Further investigation with the tracer method for CO<sub>2</sub> production will be needed to verify this observation.

### CONCLUSIONS

It can be concluded that the SF<sub>6</sub> tracer technique accurately estimated rumen CH<sub>4</sub> production. Carbon dioxide production was 20% higher by SF<sub>6</sub> tracer technique in this study. Further studies to verify this observation with CO<sub>2</sub> production are necessary. Animal-to-animal variation in CH<sub>4</sub> and CO<sub>2</sub> production was higher with the Tracer method, which implies that more animals are needed for measurements using the Tracer method than for the Cal method to determine treatment differences.

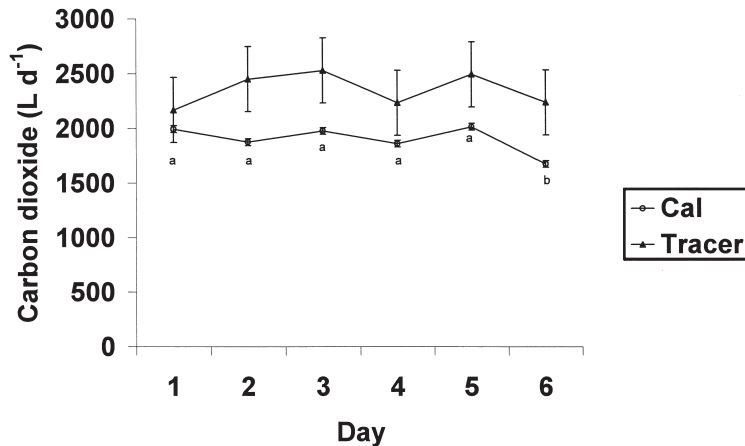


Fig. 3. Daily CO<sub>2</sub> production of heifers (mean ± SE; *n* = 3) measured by calorimetry (Cal) and SF<sub>6</sub> tracer technique (Tracer).

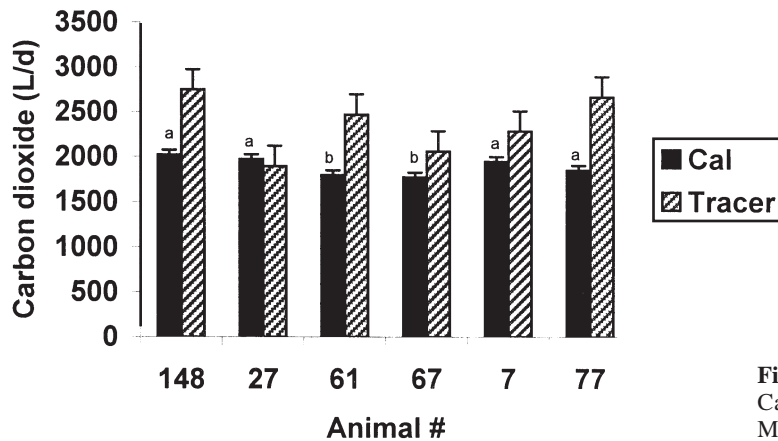


Fig. 4. Comparison of heifers' CO<sub>2</sub> production measured by Calorimetry (Cal) and SF<sub>6</sub> tracer technique (Tracer). *a*–*b* Means within a method with different letters differ (*P* < 0.05).

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